REVIEW

Cannabinoid-based therapy as a future for joint degeneration. Focus on the role of CB$_2$ receptor in the arthritis progression and pain: an updated review

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Abstract

Over the last several decades, the percentage of patients suffering from different forms of arthritis has increased due to the ageing population and the increasing risk of civilization diseases, e.g. obesity, which contributes to arthritis development. Osteoarthritis and rheumatoid arthritis are estimated to affect 50–60% of people over 65 years old and cause serious health and economic problems. Currently, therapeutic strategies are limited and focus mainly on pain attenuation and maintaining joint functionality. First-line therapies are nonsteroidal anti-inflammatory drugs; in more advanced stages, stronger analgesics, such as opioids, are required, and in the most severe cases, joint arthroplasty is the only option to ensure joint mobility. Cannabinoids, both endocannabinoids and synthetic cannabinoid receptor (CB) agonists, are novel therapeutic options for the treatment of arthritis-associated pain. CB$_1$ receptors are mainly located in the nervous system; thus, CB$_1$ agonists induce many side effects, which limit their therapeutic efficacy. On the other hand, CB$_2$ receptors are mainly located in the periphery on immune cells, and CB$_2$ modulators exert analgesic and anti-inflammatory effects in vitro and in vivo. In the current review, novel research on the cannabinoid-mediated analgesic effect on arthritis is presented, with particular emphasis on the role of the CB$_2$ receptor in arthritis-related pain and the suppression of inflammation.

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Graphic abstract
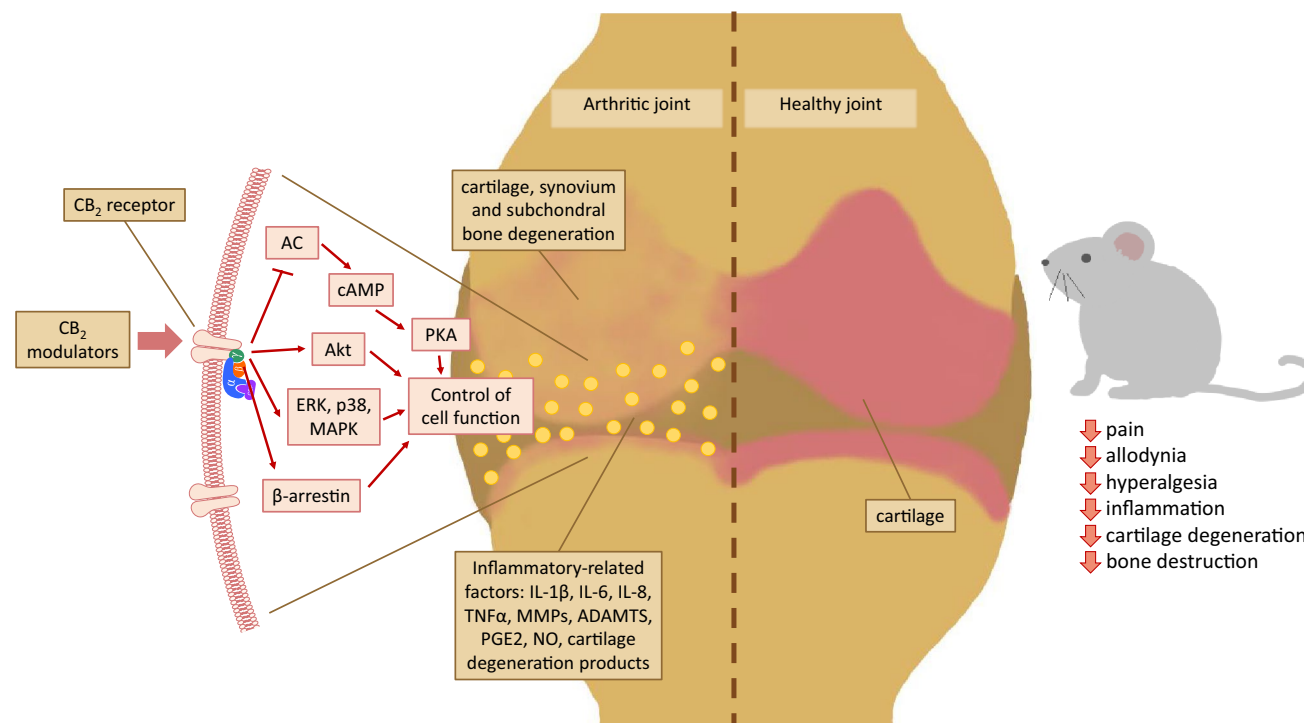
Keywords Pain · Arthritis · Cannabinoids · CB$_2$ receptor · Osteoarthritis · Rheumatoid arthritis

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
ADAMTS	Disintegrin and metalloproteinase with thrombospondin motifs
BCP	β-Caryophyllene
CB	Cannabinoid receptor
CIA	Collagen-induced arthritis
CCL2	Chemokine (C–C motif) ligand 2
COX-2	Cyclooxygenase-2
DMARDs	Disease-modifying anti-rheumatic drugs
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
FLS	Fibroblast-like synoviocytes
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GPCR	G protein-coupled receptor
GR	Glucocorticoid receptor
IL	Interleukin
JIA	Juvenile idiopathic arthritis
MAGL	Monoglycerol lipase
MMP	Matrix metalloproteinase
NSAIDs	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
OPG	Osteoprotegerin
PEA	Palmitoylethanolamide
RA	Rheumatoid arthritis
RANKL	Receptor activator of nuclear factor κB ligand
RASFs	Rheumatoid arthritis synovial fibroblasts
TLR4	Toll-like receptor 4
TNFα	Tumour necrosis factor α
TRPV1	Transient receptor potential cation channel subfamily V member 1

Introduction

The term "arthritis" refers to a widely understood joint disease that is accompanied by pain and movement limitations. However, arthritis is not an organ-specific disease. According to The Centers for Disease Control and Prevention, there are several types of arthritis, the most common of which are osteoarthritis (OA), rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), gout and fibromyalgia. In recent years, the percentage of patients suffering from OA and RA has increased due to the ageing

population and an increasing risk of diseases such as obesity and type II diabetes [1–4]. Obesity contributes to direct mechanical cartilage degeneration, because overload joint is more exposed to wear and tear damage. Arthritis, especially OA development progress as a "self-propelling wheel" with inflammation and cartilage degradation as main contributors [4]. In turn, increased risk of RA is directly related to insulin resistance and linked to systemic inflammation induced by several proinflammatory factors (e.g. TNFa, IL-6). Moreover, the prevalence of type II diabetes is also increased in patients with RA [3]. The other risk factor that may contribute to arthritis development are joint injuries [5, 6]. Musculoskeletal diseases have long-term consequences not only for patients but also for society as a whole, such as economic problems [7]. Arthritis is a leading cause of disability and one of the most common conditions among chronic users of opioids in the U.S. [8]. This condition may affect 30% of people aged 18–64 and approximately 50–60% of people over 65 years old [9]. The number of people suffering from arthritis is highly underestimated, especially among younger patients and is more common in women than in men [10]. By 2040, the number of patients reporting activity limitations due to arthritis is estimated to increase by 52% to 34.6 million people in the U.S. (11.4% of all adults) [11], which may cause vast healthcare costs.

Current therapeutic strategies focus mainly on pain attenuation and maintaining joint functionality. First-line treatments are nonsteroidal anti-inflammatory drugs (NSAIDs). However, in the advanced stages of disease, NSAIDs are not sufficient, and patients need to take stronger analgesics, such as opioids, which have undesirable side effects and may be addictive. In the most severe cases, joint arthroplasty is required to maintain patient mobility [12–14]. The abovementioned therapeutic strategies focus mainly on symptomatic treatment; therefore, the search for novel therapies is urgently needed. Cannabinoids are a promising option for pain alleviation in OA and RA not only due to their analgesic effect, but also anti-inflammatory and anti-apoptotic properties [15, 16].

Moreover, cannabinoids may help to reduce the doses of opioids used by patients to relieve pain [17]. There are two types of cannabinoid receptors (CB$_1$ and CB$_2$). Direct activation of CB$_1$ receptors may lead to adverse psychotropic side effects [18, 19], which limits their use in clinical practice. In contrast, CB$_2$ modulators seem to be safer and do not cause negative central nervous system side effects [20, 21]. Understanding of the role of the CB$_2$ receptor in pain came from the analgesic effect of the fatty acid ethanolamide–palmitoylethanolamide (PEA). The effect was blocked by the CB$_2$ receptor antagonist SR141716A [22], although PEA had no significant affinity for either CB$_1$ or CB$_2$ [23]. This finding indicates that PEA does not directly activate CB$_2$ but functions via indirect mechanisms [24].

Both OA and RA are accompanied by prolonged inflammatory states with different intensities. CB$_2$ receptors have been found on immune cells [25], which indicates that these receptors play a role in inflammatory state modulation. Chondrocytes from OA joints, even in degenerated tissue, have been indicated to express CB$_2$ receptors [26]. Moreover, CB$_2$ receptors play important roles in osteoblast migration and bone formation. Mice with inactivated CB$_2$ receptors (CB$_2$$^{-/-}$) developed osteoporosis significantly more often than control animals, while an in vitro osteoblast culture study showed that a CB$_2$ agonist promoted bone nodule formation in wild-type osteoblasts [27]. This finding demonstrates the ability of CB$_2$ receptors in joint tissues to respond to cannabinoid treatment. Several preclinical studies have demonstrated the analgesic and anti-inflammatory effects of CB$_2$ modulators in arthritis models in vivo and in vitro. The current review discusses novel research on cannabinoid-mediated analgesic effects in OA and RA, with particular emphasis on the role of the CB$_2$ receptor. The influence of cannabinoid compounds on arthritis-associated inflammation, pain and joint homeostasis are described and discussed.

Pathogenesis of arthritis

There are several types of arthritis, and OA and RA are two the most common types. A schematic changes that take place during arthritis progression are presented in the Fig. 1. Both diseases develop within the joints; however, there are some important differences between them. OA progresses slowly, leading to movement limitations and, in more severe cases, to a complete disability [28, 29]. Cartilage and subchondral bone degeneration and osteophyte formation, followed by synovial membrane inflammation (synovitis), lead to movement limitations and chronic pain [30]. Until recently, OA had been considered a "wear and tear" disease, indicating that cartilage degeneration caused by age and/or obesity was the primary cause of the disease. However, a few years ago, The Osteoarthritis Research Society (OARSI) reformulated the definition of OA, adding an inflammatory component as crucial for OA diagnosis. Joint inflammation is closely associated with macrophage influx and cytokine production, which drives the production of aggrecanases and matrix metalloproteinases (MMPs). Elevated levels of several proinflammatory factors were found in serum samples from OA patients in comparison to those of healthy individuals, but the levels were lower than those in serum samples from RA patients [31]. Interleukin 1β (IL-1β) and tumour necrosis factor alpha (TNFα) are two main proinflammatory factors produced by macrophages
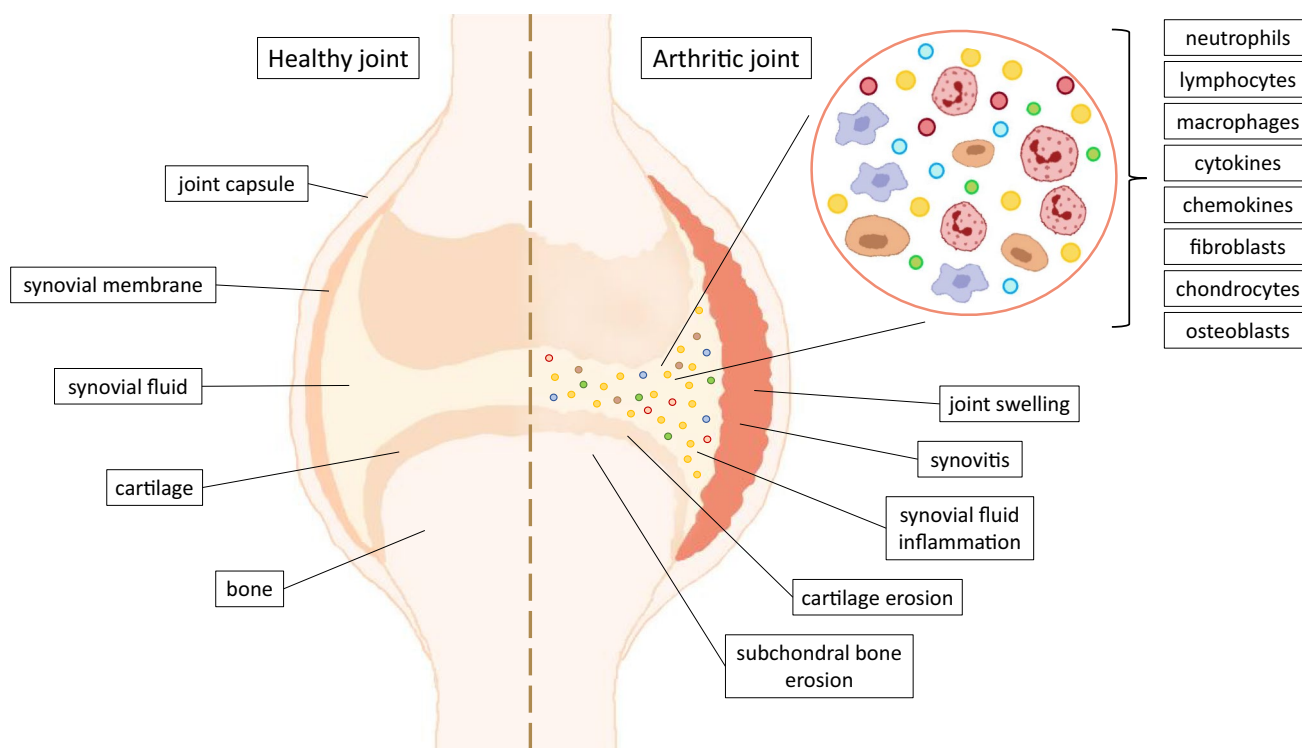
Fig. 1 Schematic summary of arthritis pathogenesis. Pathological changes, such as swelling, synovial membrane outgrowth and inflammation (synovitis), are observed in both OA- and RA-affected joints. Although the inflammatory state is an important factor in the development of OA, it is not as critical as in RA, which is a typical inflammatory disease. In the synovial fluid, several inflammatory factors, such as cytokines, chemokines, macrophages, neutrophils, fibroblasts, chondrocytes and osteoblasts, can be found. Gradual cartilage degeneration, which is characteristic of OA progression, causes bone exposure and pain, while released cartilage fragments potentiate synovitis within the joint capsule

that influence the production of other inflammatory factors [32]. Other cytokines (e.g. IL-6, IL-8, IL-15 and IL-18) and chemokines (e.g. CCL2/3/4/5/19/21 and CXCL12) also play crucial roles in synovitis and OA progression [33, 34]. Inflammatory changes can be observed not only in late OA but also in the early stages (in patients with a median age of 34 years). Favero et al. proved, that in both early- and late-stage OA an important role plays synovium-meniscus cross-talk. IL-6 and IL-8 protein levels were elevated in synovium-meniscus co-cultures from patients from both groups (with early or late-OA stage), however, the changes were higher in the late OA. In both OA stages, CCL2/MCP-1 was produced at higher levels by synovium compared with meniscus culture, while CCL5/RANTES protein release was significantly increased in co-cultures from early OA patients, in comparison to late-OA samples. MMP-3 and MMP-10 protein release was increased in both early and end-stage OA co-cultures compared to meniscus monocultures, while in the late OA the increase was approximately ten times higher in comparison to early stage. Tissue inhibitors of metalloproteinases (TIMPs) differed between groups: TIMP-2 is probably involved in the early OA, while TIMP-4 in the late stage of the disease [35]. Sohn et al. showed that Gc-globulin, a1-microglobulin and a2-macroglobulin could act via Toll-like receptor 4 (TLR4) to induce macrophage-dependent production of proinflammatory factors [31]. Thus, low-grade, prolonged inflammation is thought to be a pivotal factor in OA progression; however, a direct factor that initiates OA development is currently still unknown. Cartilage breakdown triggers the synovial membrane to release inflammatory factors into the joint space. The inflamed joint exhibit disrupted degradation-repair balance, which leads to further proteolytic enzyme production and cartilage degradation. The disease progresses in a self-perpetuating cycle in which cartilage degeneration triggers synovitis, which leads to further degradation [36]. Additionally, subchondral bone degradation seems to play a very important role in OA progression. Osteoclast-chondrocyte crosstalk, described by Hu et al., plays a significant role in joint homeostasis and OA development [37]. Since cartilage has low regenerative properties (because of the lack of innervation and blood vessels), conventional OA treatment is limited to pain attenuation and maintaining joint mobility [38]. First-line treatment is oral administration of paracetamol or NSAIDs, whereas

opioid use is necessary for severe OA pain. Intra-articular injections of corticosteroids, hyaluronic acid (viscosupplementation) or mesenchymal stem cells also provide beneficial therapeutic effects on OA patients. Additional options that support pain alleviation and maintain joint functionality are exercise and physical therapy [39]. In OA, physiotherapy is recommended as an integral part of treatment. Both aquatic and land-based exercise therapy has similar beneficial effects [40], also manual and exercise therapies similarly improve patient's mobility and there is no added benefit from a combination of these two therapies [41]. According to Juhl et al., exercise program for knee OA treatment should focus on improving aerobic capacity, quadriceps muscle strength and lower extremity performance and should be carried out three times a week [42]. In comparison to standard treatment, physical exercise may be similarly effective to hyaluronic acid injections and provide significant functional improvement in patients with moderate OA [43, 44]. In elderly OA patients, exercise increased muscle myofibrillar and sarcoplasmic protein fractional synthesis rates, whereas NSAIDs treatment reduced the level of circulating prostaglandin F2α [45]. Neuromuscular exercise effectively improved the performance of everyday activities in OA patients up to 12 months and provided greater improvement in knee symptoms (such as swelling, stiffness, etc.) in comparison do NSAID-treated group [46].

RA is an inflammatory disease characterized by persistent synovitis, systemic inflammation and autoantibodies, which lead to joint damage and disability [47]. In 2007, 1.3 million adults in the U.S. were affected by RA [48]. Women suffer from RA more frequently than men [49], and it is estimated that 1 in 12 women and 1 in 20 men will develop RA during their lifetime [50]. The main symptoms are swelling and stiffness in multiple joints (most commonly the wrists, proximal interphalangeal joints and metacarpophalangeal joints), pain and systemic symptoms (e.g. fatigue, weight loss and low-grade fever) [51]. Genetic factors are important, and the heritability of RA seems to be ~ 40% [52]. Approximately 100 specific genetic loci have been identified as associated with an increased risk of RA [53]. Apart from genetic factors, environmental factors also play a role in RA development, among which the most important are exposure to tobacco smoke, air pollution and obesity [54]. There is also evidence that imbalance in the gut microbiota and infections can contribute to RA development [55, 56]. Septic arthritis affects 2–6 per 100,000 people per year [57]. Joint inflammation is caused by bacteria, mycobacterial or fungi and the diagnosis is based on the synovial fluid analysis. The most common cause of septic arthritis is *Staphylococcus aureus*, which is responsible for 37–56% of cases [58, 59]. The study of Konig et al. proved the presence of autoantigens that are primary immune targets in RA in gingival crevicular fluid of patients with periodontal disease. They also identified a periodontal pathogen *Aggregatibacter actinomycetemcomitans* as a possible bacteria which trigger autoimmunity in RA [56].

The main factors involved in RA progression are TNFα, IL-6, IL-1, IL-17, IL-23, IL-21, IL-12, granulocyte–macrophage colony-stimulating factor (GM-CSF), CXCL8, Th2 cytokines and type 1 interferons (IFNs) [60, 61]. Circulating levels of IFN-γ, TNFα, IL-17 and IL-12 are elevated in RA patients or are produced by mitogen-stimulated peripheral blood mononuclear cells; moreover, lower levels of the anti-inflammatory factor interleukin IL-10 have been detected [62]. RA is treated with disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate, leflunomide or sulfasalazine. Early diagnosis and treatment are crucial for RA progression, and a window of opportunity may be present within the first year, especially in the first 3 months of disease onset. It was proven that very early DMARD treatment (in the first 3 months after symptom onset) gives better results than if the treatment is started after 12 months of RA symptoms [63]. However, in some patients, DMARDs are not effective. Pain in RA may arise from joint pathology and peripheral, spinal and supraspinal processing of pain signals. Sensitization mechanisms may occur at both peripheral and central levels and contribute to hyperalgesia and allodynia [64].

The endocannabinoid system

The endocannabinoid system (ECS) plays an important role in several processes, including neurodegenerative and neurological disease development [65, 66], stress-induced responses [67], pain processing [68] and immune system modulation [69], and may also link the gut microbiota and depression [70]. There are two main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and two main types of cannabinoid receptors, CB$_1$ and CB$_2$. AEA is more selective for CB1, while 2-AG has almost the same affinity for both CB receptors [71]. In addition, endocannabinoids may act via other receptors, such as transient receptor potential channels (TRPVs) or GPR receptors. Chondrocytes from patients with OA express a wide range of receptors (CB$_1$, CB$_2$, GPR55, PPRα, PPRγ). Most of receptors are highly expressed even in cells from degenerated cartilage; however, the number of chondrocytes displaying immunopositivity for GPR18 and TRPV1 is significantly decreased in degenerate cartilage [26]. Richardson et al. proved the presence of CB$_1$ and CB$_2$ protein in the synovial membrane taken from OA or RA patients; moreover, the synovial fluid of these patients contained both AEA and 2-AG, which were not detectable in healthy controls [72]. Endocannabinoids are not stored in the cell but are synthesized on demand from membrane-bound phospholipids,

and their release is vesicle-independent. In numerous brain regions, endocannabinoid signalling occur in a retrograde manner, and cannabinoid receptors are localized on presynaptic neurons [73]. At the periphery, an important role in endocannabinoid signalling plays immune system. Leukocytes and other immune cells are shown to express CB_2 and to a less extent: CB_1 receptors. Endocannabinoids in the immune system are mostly produced by macrophages, lymphocytes, astrocytes, dendritic cells, microglia and monocytes [74, 75]. Endocannabinoids play important role in the enteric nervous system of gastrointestinal tract, where CB receptors are localized on the enteric nerve terminals. In the enteric nervous system, endocannabinoids exert inhibitory actions on neurotransmission to reduce motility and secretion [76], however, this mechanism is mediated mostly via CB_1 receptors [77]. In the nervous system, CB receptors are distributed on peripheral terminals of primary afferent neurons, where play role in pain modulation: upon activation, modulate transducer ion channels and regulate neuron excitability [78].

Although AEA and 2-AG share similarities in their chemical structures, they are synthesized and degraded via two distinct Ca^{2+}-dependent enzymatic pathways. AEA is synthesized in two stages (Fig. 2). First, N-arachidonoyl phosphatidylethanolamine (NAPE) is synthetized from glycerophospholipid (GPL) and phosphatidylethanolamine (PtdEth) with the participation of calcium-dependent membrane-associated trans-N-acyltransferase (NAT) and calcium-independent NAT (RPL-1). Second, the enzymatic hydrolysis of NAPE is catalysed by NAPE-selective phospholipase D (NAPE-PLD) and leads to AEA and

phosphatidic acid production. 2-AG is produced from diacylglycerols (DAGs). Phosphoinositol bis-phosphate (PIP_2) is catalysed by PIP_2-selective phospholipase C (PLC), while phosphatidic acid (PA) is catalysed by PA phosphohydrolase (PAP), causing the formation of DAGs. Then, DAGs are converted into 2-AG by sn-1 selective-DAG lipases (DAGLs). The degradation pathway involves fatty acid amide hydrolase (FAAH)-mediated degradation of AEA and monoglycerol lipase (MAGL)-mediated degradation of 2-AG. AEA is degraded to arachidonic acid (AA) and ethanolamine (EtA), while 2-AG is degraded to AA and glycerol (G) [79, 80]. These pathways are the major pathways for the synthesis and degradation of endocannabinoids. In parallel, endocannabinoids can be synthesized and degraded via alternative pathways, and particular products may be responsible for several endocannabinoid effects. For example, AA is further metabolized on the cyclooxygenase pathway (by COX-1, COX-2) to prostaglandins, or on lipoxygenase pathway (by LOX-5, LOX-8, LOX-12, LOX-15) to leukotrienes, lipoxins and 8-, 12-, 15- hydroperoxyeicosatetraenoic acid. These metabolites might act as proinflammatory agents and generate nociceptive effects. This paradoxical effect proves ECS complexity and impede its modulation to achieve analgesic effect. It is attractive to suggest that the anti-inflammatory actions of NSAIDs are due to the COX inhibition, interestingly some NSAIDs also have the ability to inhibit FAAH (e.g. ibuprofen [81]). Moreover, FAAH inhibition might fail to induce analgesic effect due to the binding of endocannabinoids to other receptors, e.g. pro-nociceptive TRPV1. Novel dual-acting drugs, targeting endocannabinoid and endovanilloid system via interaction with FAAH enzyme together
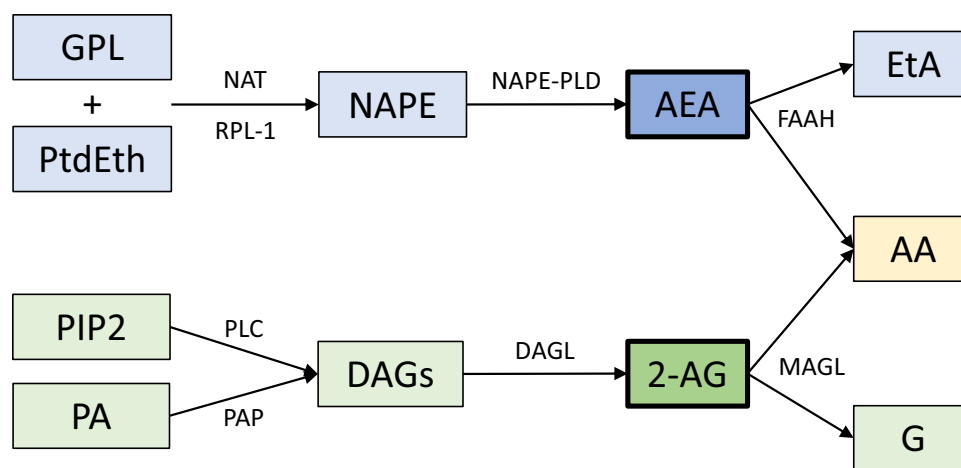

Fig. 2 The main AEA and 2-AG synthesis and degradation pathways. Abbreviations: NAT: calcium-dependent membrane-associated trans-N-acyltransferase; RPL-1: calcium-independent NAT; GPL: glycerophospholipid; PtdEth: phosphatidylethanolamine; NAPE: N-arachidonoyl phosphatidylethanolamine; NAPE-PLD: NAPE-selective phospholipase D; AEA: anandamide; FAAH: fatty acid amide hydrolase; EtA: ethanolamine; AA: arachidonic acid; PIP_2: phosphoinositol bis-phosphate; PLC: PIP_2-selective phospholipase C; PA: phosphatidic acid; PAP: phosphatidic acid phosphohydrolase; DAGs: diacylglycerols; DAGL: sn-1 selective-DAG lipases; 2-AG: 2-arachidonylglycerol; MAGL: monoglycerol lipase; G: glycerol

with TRPV1 receptor or COX-2 might lead to development of more efficient strategy for pain treatment [82, 83]. Finally, there is an evidence that AA might serve as a substrate to AEA production on the FAAH reversed pathway [84]. It was proven, that FAAH in the liver might work in reverse: in mice after partial hepatectomy, when the AA and EtA increase dramatically, FAAH activity might be switched for AEA generation form AA and EtA [85, 86]. Piscitelli and Di Marzo demonstrated that the ECS shows high redundancy, e.g. both AEA and 2-AG—besides classic route—are also inactivated through alternative biochemical routes, including hydrolysis and oxidation with several enzymes involved in this process. Moreover, endocannabinoids interact also with other than CB receptors, while the products of endocannabinoid catabolism may act on their own targets. This multidimensionality of the ECS system impedes its use in the treatment of several pathological conditions. However, knowledge of ECS redundancy could contribute to the development of novel analgesics, such as "dirty drugs" (compounds with more than one mechanism of action), and the use of certain natural products that are not currently used in the clinic [87]. Modulating the activity of the ECS seems to be a promising therapeutic strategy for several diseases, including arthritis, and is described in more detail in this review.

Cannabinoid receptors

There are two types of cannabinoid receptors: CB$_1$ and CB$_2$. Both are sevenfold membrane-bound receptors associated with G$_{i/o}$ proteins (GPCRs), and have more than one endogenous ligand (in contrast to most GPCRs) [88]. After activation, CB receptors inhibit adenylyl cyclase (AC) activity, leading to decreases in cAMP levels and stimulating mitogen-activated protein kinase (MAPK) and Akt cascades [89, 90]. Moreover, cannabinoids also act through other receptors, such as transient receptor potential channels (TRPV1, TRPV4, TRPM8 and TRPA1) and orphan receptors (GPR18 and GPR55) [91, 92]. CB$_1$ receptors are most highly expressed in the central and peripheral nervous systems, mainly in presynaptic terminals. Due to the central location of these receptors, CB$_1$ agonists induce a number of adverse effects, such as sleepiness, anxiety, euphoria and cognitive impairment, which limit their use in clinical practice. CB$_2$ receptors are located mainly in the periphery. CB$_2$ receptors were first discovered on immune cells [25, 93]; however, there is evidence of CB$_2$ expression in the central nervous system [94] such as in the cerebellum [95] or brainstem [96] and particularly on microglial cells [97]. In comparison to CB$_1$ receptors, CB$_2$ receptor expression is much less abundant; hence, it was initially thought that CB$_2$ receptors were absent from the nervous system. CB$_2$ receptors located on neurons and microglia in the spinal cord

contribute to central sensitization in OA [98]. CB$_2$ receptors might play a role in the several neurological diseases, such as Alzheimer disease [99, 100], depression [101, 102], Parkinson disease [103] or memory formation [104, 105]. However, since CB$_2$ in the other than peripheral location has been investigated recently, more studies are needed to clarify its role in various conditions.

Chondrocytes from OA joints and degenerated tissues have been proven to express a wide range of cannabinoid receptors [26]. Pajak et al. proved that the protein level of the CB$_2$ receptor increased significantly during OA progression in rat joint tissues [106]. This finding demonstrates the potential role of cannabinoids in OA treatment. The CB$_2$ receptor gene polymorphism Q63R is associated with increased arthritis risk [107, 108]. CB$_2$ is also important for the regulation of osteoblast differentiation and bone formation. Mice with inactivated CB$_2$ receptors developed osteoporosis with relative uncoupling of bone resorption from bone formation, while in primary osteoblasts from CB$_2^{-/-}$ mice, a reduced capacity to form bone nodules in vitro was observed (in wild-type osteoblast cell culture, the CB$_2$ agonist HU-308 promoted bone node formation) [27]. Mice lacking the CB$_2$ receptor suffered from more severe OA induced by surgical destabilization of the medial meniscus and spontaneous OA than WT mice [109]. In an in vitro study of human RA synovial fibroblasts (FLSs), the CB$_2$ receptor was shown to play a role in IL-1β-induced inflammation. CB$_2$ knockdown resulted in reductions in IL-1β-induced IL-6, IL-8, ENA-78, RANTES, cyclooxygenase-2 (COX-2), MMP-2 and MMP-9 production, while CB$_2$ overexpression increased IL-6, IL-8 and ENA-78 expression [110]. Additionally, CB$_2$ knockout resulted in decreased production of proteoglycans in cultures of murine articular chondrocytes in vitro compared to chondrocytes from WT animals [109]. Both synthetic [111] and plant-derived cannabinoids [112] appear to be promising candidates for pain treatment and inhibiting arthritis development.

The role of endocannabinoids in arthritis-associated pain and inflammation

Alternations in AEA have been proven to play role during neuropathic pain development [113] and OA [114]. During OA progression, AEA synthesis and degradation enzymes were elevated in the spinal cord, synovial membrane and cartilage several days in animals after OA induction [114]. AEA and 2-AG levels were augmented in the synovial fluid of dogs with OA [115]. AEA was also elevated in the joint in a posttraumatic OA mouse model [116]. In contrast to those in healthy volunteers, AEA and 2-AG have been detected in the synovial fluid of OA and RA patients [72]. Increased plasmatic levels of 2-AG and the upregulation of CB$_1$ and CB$_2$ receptor gene expression in peripheral blood

lymphocytes were also detected in OA patients compared with healthy subjects [117]. Moreover, in patients with OA, 2-AG levels are negatively correlated with leptin levels in cerebrospinal fluid, suggesting a role of 2-AG in food intake [118]. Intrathecal injection of 2-AG or AEA dose-dependently decreased carrageenan-induced mechanical allodynia in rats [119]. In in vitro bovine cartilage explants, AEA dose-dependently inhibited the release of sulphated glycosaminoglycans (GAGs) [120]. Many studies indicate the anti-inflammatory and analgesic effects of endocannabinoids. Blocking FAAH, which results in an increase in AEA, attenuates the development of arthritis and hyperalgesia in mice [121, 122]. Blocking MAGL also reduces mechanical hypersensitivity in OA rats [123]. These examples demonstrate the role of endocannabinoids and the entire ECS in arthritis pathogenesis and treatment possibilities.

CB$_2$ receptor-dependent modulation of arthritis-associated pain and progression

Mechanism of action of CB$_2$ modulators

The literature provides strong evidence for pain reduction by CB$_2$ receptor modulators in arthritis, however, the exact mechanism of action is not clear. The role of synthetic cannabinoids with higher affinities for CB$_2$ than CB$_1$ [124] in arthritis is described in detail in this review and summarized in Table 1.

A major problem of cannabis-based drugs is their non-specificity, interactions with receptors other than CB receptors and differences in effects in preclinical studies compared to clinical trials. Soethoudt et al. proved that most CB$_2$ agonists exhibit reduced selectivity regarding binding affinity and functional efficacy on mouse CB$_2$ versus CB$_1$ compared to human orthologues. Antagonists exhibited the opposite effect [125]. Moreover, the same study revealed that HU-308 induced differences in signalling effects between the human and mouse CB$_2$ receptors. This finding may indicate that HU-308 is a well-balanced agonist in all tested signal transduction pathways (GTPgS, cAMP, b-AR, pERK and GIRK)

Table 1 Binding affinities of selected synthetic and endocannabinoids; values from [124]

Compound	Ki CB$_1$	Ki CB$_2$	Classification
JWH-133	677	3.4	Selective CB$_2$ agonist
JWH-015	383	13.8	Selective CB$_2$ agonist
HU-308	> 10,000	22.7	Selective CB$_2$ agonist
WIN55,212-2	1.89–123	0.28–16.2	Non-selective CB agonist
AEA	61–543	279–1940	Endocannabinoid (nonselective)
2-AG	58.3, 472	145, 1400	Endocannabinoid (nonselective)

via human CB$_2$ receptors but is significantly biased towards G-protein activation via murine CB$_2$ receptors [125]. JWH-015 and HU-210 are agonists of GPR55 [126, 127]. JWH-015 also decreases the chemotaxis of monocytes [128]; however, it was proven to induce anti-inflammatory effects in the absence of CB$_2$ receptors, which suggests a role of noncanonical or off-target receptors. Using molecular docking and molecular dynamics analyses, Fechtner et al. showed that JWH-015 favourably bound to glucocorticoid receptor (GR), and GR knockdown reduced the anti-inflammatory effect of JWH-015 [129]. JWH-015 and JWH-133 modulate interferon α (IFNα) and TNFα responses in primary human plasmacytoid dendritic cells [130]. In an in vitro study of IL-1β-stimulated synoviocytes, JWH-133 mediated the association of CB$_2$ with TAK1 kinase to increase nuclear translocation of the transcription factors NF-κB p65 and AP-1 [110]. Most CB$_2$ modulators exert anti-inflammatory effects on arthritis animal and in vitro models [131–133]. CB$_2$ agonists can also exert paradoxical effects, which may be explained by their involvement via other receptors. For example, GW405833 reduced the mechanosensitivity of afferent nerve fibres in control joints but caused nociceptive responses in OA joints. This effect may be triggered by TRPV1 receptors, which can induce a nociceptive effect [134]. Additionally, the inhibition of enzymes responsible for endocannabinoid degradation (FAAH and MAGL) is effective in pain reduction in arthritis. URB595 (FAAH inhibitor) induces an increase in AEA, while MJN110 (MAGL inhibitor) enhances 2-AG levels. AEA and 2-AG are endogenous ligands of CB receptors, and these compounds are nonselective and show moderate binding affinities towards both receptors [125]. However, their mechanism of action depends on both CB receptors and can be blocked by specific CB$_1$ or CB$_2$ antagonists.

Analgesic and anti-inflammatory effects of CB$_2$ modulators

Several studies have revealed the analgesic potential of CB$_2$ receptor modulators, whereas in vitro models have confirmed the anti-inflammatory and anti-degenerative effects of CB$_2$-targeting compounds. In collagen-induced arthritis (CIA) in mice, CB$_2$ receptor expression was markedly higher in arthritic animals than in the control group [135]. JWH-133 suppressed CIA, synovial hyperplasia, inflammatory responses, cartilage damage and bone destruction. Moreover, JWH-133 repolarized the macrophage phenotype from M1 to M2, promoted anti-inflammatory IL-10 expression and diminished TNFα, IL-1β and IL-6 levels [135]. Fukuda et al. proved that JWH-133 reduced arthritis scores, inflammatory cell infiltration, bone destruction and anti-CII IgG1 production in CIA mice [136]. In sodium monoiodoacetate (MIA)-induced OA, systemic administration of JWH-133 reduced pain, inflammation, spinal astrogliosis and MMP-2 and MMP-9 activity in treated rats. In turn, spinal JWH-133

administration diminished noxious-induced responses in spinal neurons [98]. OA mice with CB$_1$ knockout show effective manifestations that are not observed in CB$_2$-KO mice. JWH-133 and ACEA (CB$_1$ agonist) ameliorated nociceptive and affective alterations [117]. In healthy rats, JWH-133 increased synovial blood flow, and the effect was blocked by the CB$_2$ receptor antagonist AM630 and the TRPV1 receptor antagonist SB366791. This finding indicates the role of the TRPV1 receptor in the mechanism of action of JWH-133. In arthritic animals, the vasodilatory effect of JWH-133 was diminished [137]. JWH-133 also exerts anti-inflammatory effects in vitro. JWH-133 reduces IL-6, MMP-3 and CCL2 production in TNFα-stimulated RA/OA FLSs [136]; however, the pretreatment of RA FLSs with JWH-133 before inflammatory stimulation did not reduce the IL-1β-induced increase in IL-6 and IL-8 expression and augmented COX-2 expression [110]. JWH-133 also increased osteoclast formation in osteoblast-bone marrow co-cultures in vitro [138].

The receptor activator of nuclear factor κB ligand/osteoprotegerin ratio (RANKL/OPG) is an important indicator of bone homeostasis and remodelling. This ratio determines osteoclast proliferation and activity and, therefore, controls bone formation and resorption [139]. In RA patients, the RANKL/OPG ratio was significantly decreased [140]. JWH-015, another CB$_2$ agonist, ameliorated pain in arthritic rats, inhibited bone destruction, increased RANKL and decreased OPG levels [129]. JWH-015 also increased synovial blood flow in healthy animals [137]. In human RA FLSs in vitro, JWH-015 inhibited the ability of IL-1β to induce the production of IL-6, IL-8 and COX-2; however, this effect was partially mediated by the GR receptor, since the effect of JWH-015 persisted after CB$_2$ knockdown [129].

Similar to JWH-133, HU-308, a selective CB$_2$ agonist, reduced osteoclast formation in osteoblast-bone marrow co-cultures [138]. In IL-1β-, TNFα- or LPS-stimulated OA/RA FLSs, HU-308 diminished FLS proliferation but also inhibited MMP-3, MMP-13, IL-6 production and IL-1β-induced activation of extracellular ERK1/2 and p38 MAPK [141]. In LPS-stimulated mouse peritoneal macrophages from WT mice, HU-308 decreased the levels of IL-6 and TNFα but had no effect on macrophages from CB$_2$-KO mice, suggesting the participation of the CB$_2$ receptor in the mechanism of action of HU-308 [132]. The same study revealed that in the CIA mouse model, HU-308 did not inhibit the incidence of CIA development but alleviated the severity of CIA and decreased joint swelling, synovial inflammation, joint destruction and serum levels of anti-collagen II antibodies [132]. In surgically induced OA in CB$_2$-KO and WT mice, HU-308 reduced OA severity. More severe OA was observed in CB$_2$-KO mice than in WT mice [109].

WIN55,212-2, a nonselective CB agonist, has been widely studied in vitro, and its anti-inflammatory and anti-degradative properties have been verified. In TNFα-stimulated RA FLSs, WIN55,212-2 showed dose-dependent anti-inflammatory effects. At low concentrations, WIN55,212-2 decreased IL-6, IL-8 and MMP-3 production (an effect independent of CB$_1$ or CB$_2$ activation, but attenuated by TRPV1 or TRPA1 inhibition) and increased FLS adhesion. While higher concentrations of WIN55,212-2 reduced IL-6, IL-8 levels, adhesion and proliferation of FLS, it increased extracellular MMP-3 level [133]. In primary human OA articular chondrocyte cultures, WIN55,212-2 mesylate inhibited the activity of a disintegrin and metalloproteinase with thrombospondin motif 4 (ADAMTS-4, a major contributor to the pathogenesis of OA) and syndecan-1 expression, which suggests an antiarthritic effect [142]. WIN55,212-2 also regulates the gene and protein levels of MMPs and tissue inhibitors of metalloproteinases (TIMPs). MMP-3, MMP-13, TIMP-1 and TIMP-2 gene expression and MMP-3 and MMP-13 protein secretion were decreased after WIN55,212-2 treatment in IL-1β-stimulated chondrocyte cultures [143]. WIN55-212-2 administration in both IL-1α-stimulated bovine chondrocytes and explants, reduced proteoglycan, collagen degradation, iNOS, COX-2 expression and NF-κB activation. But the reduction in NO production was observed only in IL-1α-stimulated bovine articular chondrocytes which potentiated by AM281 and AMM630. Also WIN55,212-2-induced decrease in sulphated glycosaminoglycans was reported [120, 144]. WIN55-212,2 was also proven to decrease IL-6 and IL-8 expression; however, this effect was not inhibited by CB$_1$ or CB$_2$ antagonists, which indicates a CB receptor-independent mechanism of action. A similar effect occurred with the nonselective CB agonist CP55,940 [145], which was also proven to stimulate osteoclast formation in vitro [146].

Another selective CB$_2$ agonist, A-796260, increased grip force in a rat OA model [147]. 4-Quinolone-3-carboxamide (4Q3C) was proven to reduce bone erosion, joint destruction, osteoclast formation and the general severity of arthritis in mice. Moreover, the same study revealed that 4Q3C diminished the proinflammatory factors TNFα, IL-1β and COX-2 and the RANKL/OPG ratio [131]. GW405833 reduced the joint afferent firing rate in control animals but caused nociceptive responses in OA joints and increased hindlimb incapacitance in rats. This effect was diminished by the CB$_2$ receptor antagonist AM630 and the TRPV1 receptor antagonist SB366791, which proves that lack of selectivity of GW405833 and the involvement of TRPV1 receptors in this mechanism of action [134]. The plant-derived CB$_2$ agonist β-caryophyllene (BCP) alleviated the severity of collagen antibody-induced arthritis, deceased MMP-3 and MMP-9 expression in joints, reduced proinflammatory cytokine expression and increased anti-inflammatory cytokine expression [112]. The studies described in this paragraph are summarized in Table 2.

Table 2 Effects of selected cannabinoids on the regulation of pain and inflammation in arthritis models in vivo and in vitro

Compound	Model	Key findings	References
JWH-133	CIA mouse model	↓ TNFα, IL-1β, IL-6, synovial hyperplasia, cartilage damage, bone destruction, M1-like macrophages, osteoclast formation, osteoclastic bone resorption, RANKL-induced NF-kB activation in the osteoclast precursors ↑ IL-10	[135]
	MIA-induced OA rat model	Systemic administration: ↓ pain, inflammation, spinal astrogliosis, MMP-2, MMP-9 activity Spinal administration: ↓ noxious-evoked responses of spinal neurons	[98]
	TNFα-stimulated; RA/OA FLS and CIA mouse model	In vivo: ↓ IL-6, MMP-3, CCL2 In vitro: ↓ arthritis score, inflammatory cell infiltration, bone destruction and anti-CII IgG1 production	[136]
	IL-1β-stimulated human RA FLS	JWH-133 pretreatment: no reduction of IL-1β-induced IL-6 and IL-8 production, ↑ COX-2	[110]
JWH-133 or ACEA	CB₁KO, CB₂KO and WT mice with MIA-induced OA	CB₁KO mice: ↑ arthritis affective manifestations JWH-133: ↓ nociceptive and affective OA alterations ACEA: ↓ nociceptive and affective OA alterations, ↑ memory	[117]
JWH-133 or JWH-015	Kaolin/carrageenan-induced or Freund's adjuvant arthritis rat models	JWH-133 and JWH-015 in control animals: ↑ synovial blood flow (JWH-133's effect blocked by AM630 or SB366791) Arthritic animals: ↓ of vasodilatory effect of JWH-133	[137]
JWH-015	IL-1β-stimulated RA FLS and rat adjuvant-induced arthritis model	In vitro: ↓ IL-6, IL-8, COX-2, phosphorylation of TAK1 and JNK/SAPK (still effective after CB₂ knockdown) In vivo: ↓ arthritis, pain, bone destruction, RANKL level; ↑ OPG level	[129]
JWH-133 or HU-308 (in vitro only) or AM630	Osteoblast-bone marrow co-cultures and RANKL- and M-CSF-generated osteoclasts; CB₂KO and WT mice	In vitro: JWH-133 and HU-308: ↑ osteoclast formation AM630: ↓ osteoclast formation and activity In vivo: AM630: protected against bone loss in WT, but not CB2-/- mice	[138]
HU-308	IL-1β, TNFα- or LPS-stimulated FLS from OA or RA patients	↓ FLS proliferation, MMP-3, MMP-13, IL-6 production, IL-1β-induced activation of extracellular ERK 1/2 and p38 MAPK	[141]
	CIA mouse model; LPS-stimulated mouse peritoneal macrophages from WT or CB₂KO mice	In vivo: no inhibition of incidence of the development of CIA, ↓ severity of CIA, joint swelling, synovial inflammation, joint destruction, serum levels of anti-collagen II antibodies In vitro: ↓ IL-6, TNFα (no effect on macrophages from CB₂KO mice)	[132]
	Surgically-induced or spontaneous OA in CB₂KO or WT mice	More severe OA in CB₂KO mice HU-308: ↓ severity of OA	[109]

Table 2 (continued)

Compound	Model	Key findings	References
WIN55,212-2	TNFα-stimulated FLS from OA and RA patients	Low concentrations: ↓ IL-6, IL-8, MMP-3 production (effect independent on CB_1 or CB_2 activation, but attenuated by TRPV1 or TRPA1 inhibition); ↑ FLS adhesion. High concentrations: ↓ IL-6, IL-8; ↑ extracellular MMP-3 (effect decreased by BAPTA, metformin, A967079 and COR170); ↓ FLS adhesion and proliferation	[133]
	IL-1β-stimulated human OA chondrocytes	↓ ADAMTS-4 activity (effect abolished by JTE907, but not MJ15) ↓ expression of syndecan-1 (overexpression of syndecan-1 reversed the inhibitory effect of WIN-55 on the ADAMTS-4 activity)	[142]
	IL-1β-stimulated human OA chondrocytes	↓ MMP-3, MMP-13, TIMP-1, TIMP-2 gene expression ↓ MMP-3, MMP-13 protein production	[143]
	IL-1α-stimulated bovine articular chondrocytes and cartilage explants	↓ NO production in chondrocytes (effect potentiated by AM281 and AM630) ↓ release of sulphated glycosaminoglycans in cartilage explants	[120]
WIN55,212-2 or HU-210	IL-1α-stimulated bovine chondrocytes and explants	WIN55,212-2: ↓ proteoglycan and collagen degradation, iNOS, COX-2 expression, NFκB activation HU-210: ↓ proteoglycan and collagen degradation	[144]
WIN55,212-2 or CP55,940	IL-1β-stimulated RA and OA FLS	WIN55,212-2 and CP55,940: ↓ IL-6, IL-8 expression (not inhibited by CB_1 nor CB_2 antagonists)	[145]
A-796260	MIA-induced rat OA model	↑ grip force	[147]
4Q3C	CIA mouse model	↓ arthritis severity, histopathological changes, bone erosion, osteoclast formation ↓ RANKL/OPG ratio, TNFα, IL-1β, COX-2, NO expression	[131]
GW405833	MIA-induced rat OA model	Control animals: ↓ joint afferent firing rate OA animals: ↑ sensitization of mechanoreceptors (diminished by AM630 or SB366791), ↑ hindlimb incapacitance, ↑ CGRP release	[134]

Compound classification: 4Q3C: CB_2 agonist; A-796260: CB_2 agonist; ACEA: CB_1 agonist; AM251: CB_1 antagonist; AM630: CB_2 antagonist; AM281: CB_1 antagonist; AM630: CB_2 antagonist; BAPTA: calcium chelating agent; COR170: CB_2 antagonist; CP55,940: nonselective CB agonist; GW405833: CB_2 agonist; HU-210: nonselective CB agonist; HU-308: CB_2 agonist; JTE907: CB_2 antagonist; JWH-015: CB_2 agonist; JWH-133: CB_2 agonist; metformin: AMPK activator; MJ15: CB_1 antagonist; rimonabant: CB_1 antagonist; SB366791: TRPV1 antagonist; SR144528: CB_2 antagonist; N55,212: nonselective CB agonist

Attenuation of OA pain and inflammation by inhibiting endocannabinoid degradation

Apart from the administration of CB_2 agonists, ECS modulation may also be achieved via the inhibition of AEA or 2-AG degradation. Blocking the enzyme FAAH results in prolonged local AEA accumulation. One of the best studied FAAH inhibitors is URB597. Kinsey et al. proved that prolonged FAAH inhibition (in genetically modified $FAAH^{-/-}$ mice or after repeated URB597 administration) reduces the severity of CIA. Decreases in hyperalgesia and the severity of CIA in $FAAH^{-/-}$ mice were reversed by chronic administration of the CB_2 receptor antagonist SR144528, while the effect of URB597 was prevented by the acute administration of the CB_1 receptor antagonist rimonabant. This finding suggests that prolonged CB_2 receptor activation diminishes CIA severity, whereas acute CB_1 receptor activation reduces hyperalgesia in a CIA model [121]. In the kaolin/carrageenan-induced joint inflammation model, low doses of URB597 reduced hyperaemia and leukocyte rolling and adhesion. Moreover, improvements in hindlimb weight bearing and withdrawal thresholds were observed, which were mediated by CB_1 receptors. Importantly, the effects on leukocyte rolling and hyperaemia were blocked by both CB_1 and CB_2 antagonists, while the reduction in leukocyte adhesion was independent of CB receptor activation [148]. In two rodent OA models, Schuelert et al. proved that peripheral administration of URB597 significantly reduced the afferent firing rate and hindlimb incapacitance (the effect was blocked by the CB_1 receptor antagonist AM251), while local URB597 injection reduced mechanonociception and pain (this effect was mediated by CB_1 receptors) [149]. Because FAAH inhibitors did not exhibit sufficient efficacy in clinical trials (despite their analgesic effects in animal studies), the efficacy of dual-acting FAAH and TRPV1 inhibitor (OMDM-198) in pain reduction was tested. OMDM-198 showed anti-hyperalgesia effects in an OA animal model. The effectiveness was comparable to that of a selective TRPV1 antagonist (SB-366,791) and a selective FAAH inhibitor (URB597) and was blocked by AM251 and olvanil (TRPV1 inhibitor) [83].

In turn, MAGL blockade results in the inhibition of 2-AG degradation. In the MIA-induced OA rat model, acute MJN110 administration was proven to significantly elevate brain 2-AG levels. A single injection of MJN110 restored weight-bearing asymmetry and lowered the withdrawal threshold. Both effects were blocked by SR144528, a CB_2 receptor antagonist, while CB_1 blockade by SR141716A inhibited only the restoration of weight-bearing asymmetry. In the same study, repeated MJN110 administration resulted in antinociceptive tolerance at a high dose (5 mg/kg); however, a low (1 mg/kg) dose reduced pain but did not alter joint histology. MJN100 inhibited the expression of

membrane-associated PGE synthase-1 in the spinal cords of OA rats [150]. Another MAGL inhibitor, KLM29, reduced pain in the MIA-induced OA rat model, and this effect was blocked by CB_1 and CB_2 antagonists. The COX-2 inhibitor celecoxib was also administered, a significant reduction in joint pain and inflammation was noticed and mechanical allodynia development was prevented in the later OA stages [123]. Studies on the analgesic effect of selected inhibitors of endocannabinoid-degrading enzymes in arthritis are summarized in Table 3.

Future prospects

The studies presented in this review confirm the role of the ECS in arthritis pathogenesis and the efficacy of both endocannabinoids and novel synthetic CB_2 modulators in pain management. Chronic pain of various origins is currently a global problem. In more severe cases, patients are forced to use stronger analgesics such as opioids, which are effective in alleviating pain but have a high potential for addiction. Opioid use disorder symptoms are increasingly diagnosed in chronically ill patients. Cannabinoid-based opioid replacement therapy may be an analgesic alternative that can help to ease opioid withdrawal symptoms and decrease the likelihood of addiction relapse [151]. In chronic and inflammatory pain models in rodents, combined mu opioid receptor (MOR) and CB_2 receptor agonism resulted in significant synergistic pain inhibition and reduced opioid-induced side effects [152]. In turn, La Porta et al. proved that after OA induction, CB_1KO and CB_2KO animals developed allodynia at similar levels to wild-type mice, whereas in CB_2xP transgenic animals (overexpressing CB_2 receptors), allodynia was significantly attenuated. Moreover, the role of functional interactions between the ECS and the opioid system in the control of joint pain has been described [153]. These data suggest that cannabinoids can be an efficient alternative for opioids and reduce the opioid doses used by patients with chronic pain.

Apart from the abovementioned advantages of cannabinoids in chronic pain, ECS modulation itself might be a useful strategy for treating arthritis and the accompanying pain and inflammation. Although endocannabinoids are not selective for the CB_2 receptor, they have been proven to diminish hyperalgesia in various arthritis animal models and prevent joint damage. AEA or 2-AG degradation inhibitors in combination with other currently available treatment strategies, such as the coadministration of COX-2 inhibitors, may give improved results in both pain alleviation and anti-inflammatory effects [82].

Cannabinoids not only alleviate joint hyperalgesia but also may help to prevent joint damage, chronic pain development and disease progression [121]. In addition to endocannabinoids, synthetic CB_2 modulators exert analgesic

Table 3 Effects of selected inhibitors of endocannabinoid-degrading enzymes on the regulation of pain and inflammation in arthritis models in vivo

Compound	Model	Key findings	References
URB597	CIA mouse model in WT, FAAH$^{-/-}$ and FAAH-NS (expressing FAAH exclusively in nervous tissue)	FAAH$^{-/-}$ and FAAH-NS: ↓ CIA severity (blocked by SR144528) Repeated URB597 ↓ CIA severity (blocked by rimonabant) Acute URB597: ↓ hyperalgesia (blocked by rimonabant)	[121]
	Kaolin/carrageenan joint inflammation mouse model	↓ hyperaemia (blocked by AM251 and AM630) ↓ leukocyte rolling and adhesion (independent of CB receptors activation) ↑ hindlimb weight bearing, withdrawal thresholds (blocked by AM251)	[148]
	MIA-induced rat OA and spontaneous OA guinea pig models	Peripheral administration: ↓ afferent firing rate, hindlimb incapacitance (blocked by AM251) Local administration: ↓ mechanonociception and pain (blocked by AM251)	[149]
MJN110	MIA-induced OA rat model	Acute MJN110: ↓ weight-bearing asymmetry (blocked by SR141716A and SR144528), withdrawal threshold (blocked by SR144528) Repeated MJN110: ↓ PGE synthase-1, ↑ 2-AG brain level; 5 mg/kg: antinociceptive tolerance; 1 mg/kg: ↓ pain, no effect on joint histology	[150]
KML29 alone or with celecoxib	MIA-induced OA rat model	KML79 alone: ↓ pain (blocked by AM281 and AM630) KML29 + celecoxib: ↓ withdrawal threshold, leukocyte trafficking (in acute inflammatory phase), ↓ mechanical allodynia (later OA stages)	[123]

Compound classification: AM251: CB$_1$ antagonist; AM281: CB$_1$ antagonist; AM630: CB$_2$ antagonist; celecoxib: COX-2 inhibitor; KML29: MAGL inhibitor; MJN110: MAGL inhibitor; rimonabant: CB$_1$ antagonist; SR141716A: CB$_1$ antagonist; SR144528: CB$_2$ antagonist; URB597: FAAH inhibitor; URB937: FAAH inhibitor

and anti-inflammatory effects in various in vitro and in vivo arthritis models [133, 154, 155]. However, cannabinoid therapy is still not widely used. One of the main drawbacks of cannabinoids is their psychoactive component, which is mainly associated with CB$_1$ receptors, which are localized mostly in the central nervous system. The peripheral location of the CB$_2$ receptor, especially on immune cells, makes it a better therapeutic target, with very limited side effects. CB$_2$ modulators, which have been proven to have analgesic and anti-inflammatory properties with no or mild negative side effects might be an interesting alternative to NSAID therapy, especially in the early, non-severe arthritis stages. Although there are indications for effective arthritis therapy, further preclinical studies need. Despite the fact that arthritis is a common disease that affects an increasing number of patients every year, the endocannabinoid approach to treatment is still not very popular in therapeutic practice. Cannabinoid-based drugs (especially those targeting CB$_1$ receptors) possess dose-limiting side effects and may have modest clinical efficacy. However, increasing interest in products containing cannabinoid extracts has been observed in recent years, which may lead to the use of cannabinoids on a larger scale but might also carry the risk of overdose and the treatment of cannabinoids not as drugs but as harmless dietary supplements. As of today, clinical trials with synthetic CB$_2$ modulators in arthritis are still limited, nevertheless, in vivo studies offer hope for an effective clinical therapy in the future.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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